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EXPERIMENTS WITH "STINGLESS BEES" (*TRIGONA CRESSONI PARASTIGMA*) CONCERNING THEIR ABILITY TO DISTINGUISH ULTRAVIOLET PATTERNS

BY FRANK E. LUTZ

Several years ago it was demonstrated[1] that many flowers reflect the ultraviolet of sunlight and, so, have an ultraviolet color in the eyes of any creature that can see ultraviolet, just as many flowers reflect the blue of sunlight and, so, have a blue color in our eyes. It was also shown that all of the flower-visiting insects that were tested could see the ultraviolet of sunlight transmitted through a filter that cut out that part of the spectrum which is visible to us. More recently it has been found[2] that many butterflies and moths have ultraviolet color-patterns. Accordingly, a better knowledge concerning the ability of insects to distinguish ultraviolet color and color-patterns is highly desirable.

The experiments with flower-visiting insects were conclusive as to the ability of those insects to perceive and react to ultraviolet, but the experiments did not show that the insects differentiate ultraviolet in their natural environment and react to it in a normal way. The apparatus used was a box into which the insects were placed and from which they tried to escape through one or more windows screened with light-filters. Presumably they would try hardest at the window which looked brightest to them and that was the window which transmitted the ultraviolet of sunlight but which looked dark to us because it did not transmit the light visible to us.

The Von Frisch school of experimentalists in Munich has secured exceptionally interesting results[3] concerning a variety of points in the psychology and physiology of the domestic bee, *Apis mellifera*. The principal method of procedure has been to accustom honey-bees to come to a definite place for honey or sweetened water and then to associate with that food some color or pattern or whatever was being studied.

[1] Lutz, F. E., and Richtmyer, F. K., 1922, Science, LV, May 12. Richtmyer, F. K., 1923, Journ. Optical Soc. Amer., VII, pp. 151–168. Lutz, F. E., 1924, Annals New York Acad. Sci., XXIX, pp. 233–279.

[2] Lutz, F. E., and Burlingame, R., 1932, Report, as yet unpublished, at the Atlantic City meeting of the Entomological Society of America.

[3] See, for example, Baumgärtner, H., 1928, 'Der Formensinn und die Sehschärfe der Bienen,' Zeit. f. Verg. Physiol., VII, pp. 56–143.

After a short period of training in this association the bees were tested in order to discover how accurately they could distinguish the associated thing from things not thus associated.

Anticipating a short stay at the Barro Colorado Island Laboratory of the Institute for Research in Tropical America, I prepared to try the same technique with one or more of the several species of *Trigona* that are fairly abundant there, largely to see if they furnished good material for such work but also hoping that at least some information might be obtained concerning the reactions of a completely feral species in its natural environment to ultraviolet color.

These *Trigona* belong to the tropical family Meliponidae, the so-called Stingless Bees. They are social but are apparently not very closely related to our common honey-bee, an Asiatic species of the family Apidae. Much to my surprise, I found, when attempting to start the work, that the *Trigona* then present in the Laboratory clearing paid no attention to sweetened water, even when it was placed close to their nest-opening. I tried both refined, white sugar and the native, very brown kind; also honey, maple syrup, sweetened orange syrup, grenadine, and ripe fruits. Knowing that certain species eat meat to some extent, I tried that, lard, and other substances without success. The only thing that I found to interest any of the bees at that time and place was fecal matter. They collect such excrement, probably for use in nest-building. However, this attractant is neither pleasant nor convenient to use.[1] Therefore, I took the somewhat desperate chance of bees forming an association between nest and color or color-pattern that would be stronger than their sense of nest-location based on other environmental features.

There was a colony of *Trigona cressoni parastigma* that had its nest in a wall of the laboratory building. The bees used a part of the space between the adjoining ends of two boards as a passage-way to and from the nest. There was no nest-entrance "funnel" such as is frequent on *Trigona* nests but, except for about 15 mm. used as a passage-way, the space between the boards was neatly filled with a black, waxy mixture flush with the surface of the boards. As was to be expected, most of the bees flew directly away from and to this nest-entrance as though its position on the side of the building were well-marked and thoroughly known. From time to time one or more bees, probably young ones, flew back and forth a meter or so from the building as though making

[1] As a matter of fact, experiments were started with excrement as an attractant for *Trigona pallida* but they were not carried to any definite conclusion. Also, several days before the end of my stay a swarm of *Trigona testacea cupira* moved into the Laboratory clearing and individuals of this swarm were attracted to sugar and fruits.

"orientation observations." The principal large objects by which they might orient themselves were a vertical water-pipe two or three meters to the left and a flight of outside stairs about the same distance to the right of the nest entrance.

Experiments with a large variety of Hymenoptera have shown how remarkably exact is their knowledge of the location of their nest. In some cases the fact that small objects in the immediate vicinity of a nest are used as "markers" is shown by the confusion caused by disturbing these objects. In other cases it appears that more general features of the nest's environment are used or else—less probable—that some mysterious "location sense" is involved. For example, if the hive of a colony of *Apis mellifera* be moved only a few centimeters, the bees that were absent when the shift occurred will return to the old location although the hive is probably plainly visible to them and might be expected to act as an important landmark.

In the Munich experiments on the vision of *Apis* the dish or box containing the attractant could be moved every few minutes so that the bees formed no definite location association. This, of course, was impossible with the *Trigona* when using the nest itself as the attractant. This condition proved to be a real difficulty but the results obtained seem to be all the more conclusive because of it.

Another desirable feature of the Munich experiments that could not be incorporated in the present ones was the marking of individual bees so that the observer might know which had been subjected to "training" and for how long. When food is used as an attractant, it can be so arranged that only a few individual bees are concerned. Here we had the whole colony, including those which matured during the course of the work.

The plan of the present experiments was to place a color or color-pattern around the nest opening with contrasting colors or color-patterns in its immediate vicinity. Naturally, the position of the nest-marking color or color-pattern could not be shifted during the time that the bees were being "trained" to associate it with their nest-opening. However, the positions of the other colors or color-patterns were shifted frequently—usually every hour. This was done in an attempt to prevent, so far as possible, the bees from associating the location of their nest with the positions of the colored discs in general.

In order to facilitate the shifting of the colored cards, folders of heavy Manilla paper were made to contain them. The folders were about ten centimeters square. There was a circular hole about fourteen millimeters

in diameter in the center of each colored card and in the back of each folder. There was also a centered circular hole about six and a quarter centimeters in diameter in the front of each folder, exposing the colored card. By an arrangement of strings these folders with the enclosed cards could be hung against the side of the building.

For the sake of easy reference a "map" of the nest-vicinity was divided into squares and lettered as shown in figure 1. "M" was the nest-opening square. When a folder and its contained card was placed,

A	B	C	D	E
F	G	H	I	J
K	L	M	N	O
P	R	S	T	U
V	W	X	Y	Z

Fig. 1.—A "map" of the spaces upon which experimental patterns were placed.

on M, the nest-opening showed as a vertical black streak in the central hole of the card. Accordingly, a black streak was painted on the side of the house wherever a folder with its card was to be hung.

Training in Associating Color-pattern with Nest-location

In order to determine whether these bees would reasonably soon come to associate the location of their nest with an at least striking color-pattern as opposed to an equally striking, but different, color-pattern and other features of the nest's vicinity, I put *d* of figure 2 at the nest opening (space M). This pattern consists of four black sectors and alternating with them four white sectors that reflect ultraviolet—that is, are ultraviolet in addition to the colors we see. Pattern *a* of figure 2

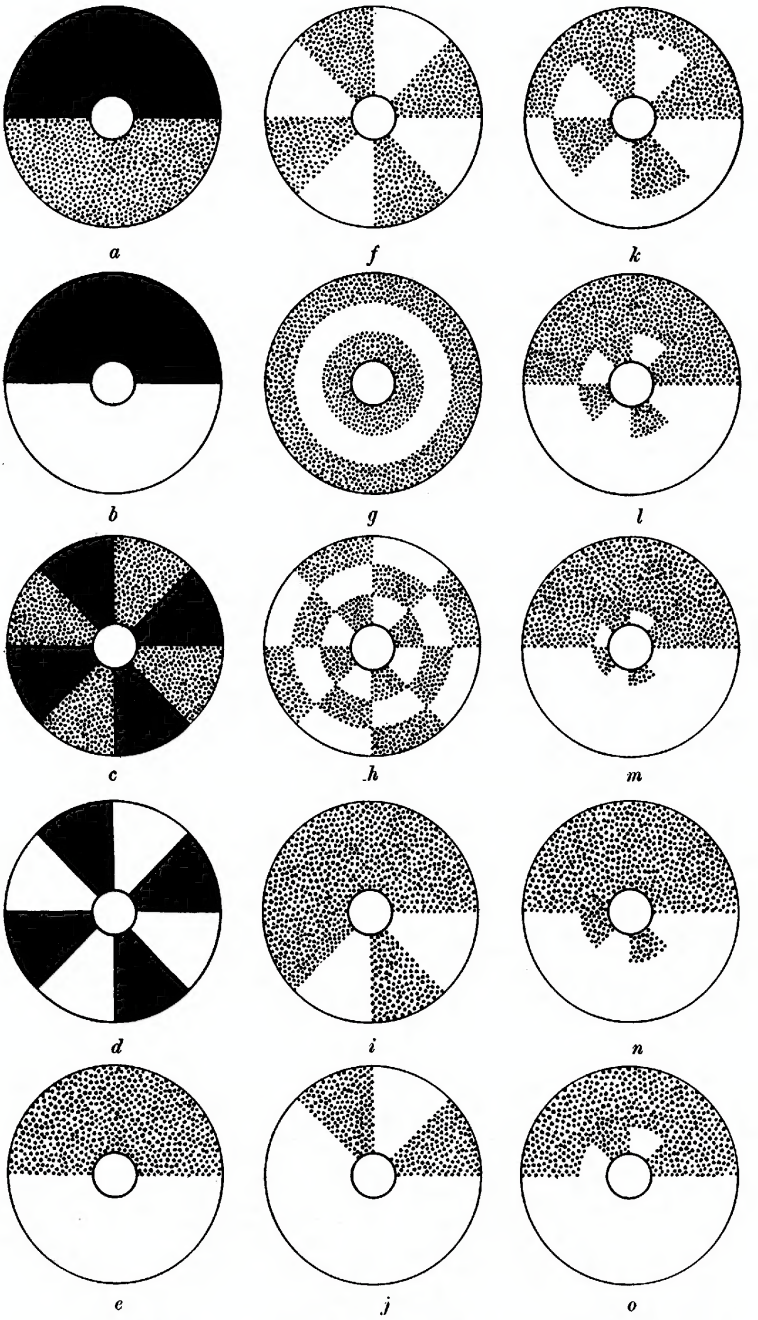


Fig. 2. Diagrams of patterns used. Stippled areas were white with little or no ultraviolet. Unshaded areas were white reflecting ultraviolet.

was used in contrast with it. This pattern consists of a semicircle of black above; below is a semicircle of white (Chinese White) that does not reflect ultraviolet.

Starting at 1:45 P.M., March 4, there was a folder with a *d* card at M (the nest) and folders with *a* cards at H and N. At 8:00 A.M. the next day the folders with *a* cards were moved to G and L, and at 11:00 A.M. to S and T. It will be noted by reference to figure 1 that in each of these arrangements the three cards, taken together, formed a right angle like the letter L and that the shifts brought the nest opening successively to each of the three relative positions in the "L."

It is difficult to tabulate or even to describe in a few words the behavior of the incoming bees. This difficulty is of importance when scoring the "tests" in which the nest-marking pattern is put where the nest-opening is not. Under normal conditions the bees may go directly to the opening, usually sweeping more or less upward from below, or they may hover about half a meter in front of it before darting in. Under the artificial conditions of this experiment when a bee mistook the central hole of an *a* card for its nest-opening, it might discover its error before actually alighting. In that case it usually flew back a bit and tried again, often at the same card. Such darting toward the card, even if not actually alighting, was at first counted as a selection of that card; but, as will be seen, the system of scoring was changed several times in an effort to get a good one.

Starting at 2:00 P.M. (March 5), with the *d* card still at M (the nest opening) and with *a* cards still at S and T, the following selections were made by twenty returning bees, each bee's record being separated by a period and dash: T, T, S, M.—T, M.—M.—M.—S, M.—S, T, M.—M.—S, then flew away.—M.—S, T, S, T, S, M.—T, S, M.—M.—S, M.—M.—M.—T, S, M.—S, S, M.—M.—M.—S, S, T, T, T, M. Of the twenty, 9 went straight to the nest (M with the *d* card); 7 went first to the *a* card on S; and 4 went first to the *a* card on T. Since there were two possibilities of a false choice (the *a* cards on S and T) and only one that was correct (the *d* card on M), the "first choice" of these bees was correct somewhat more often (9 in 20) than it would be (6.7 in 20) if a purely random selection had been made. Of course, having made the correct choice, the bee did not look elsewhere; but if it made a wrong choice, it either tried until the correct one was made or it flew away. The frequent choice of the card on S in this set of observations is not to be explained by its position in relation to the other cards, since the angle of the "L" formation had not recently been the position of the nest. It will

be noted in the records of subsequent observations that a card on S is often favored. Possibly this is because S is just below the nest and in the same vertical line in space.

The position of the cards not having been changed, the choices of another twenty bees were recorded starting at 3:30 P.M. of the same day. They were as follows: M.—S, M.—M.—S, T, M.—M.—T, and away.—M.—M.—S, M.—M.—M.—M.—S, M.—S, M.—M.—M.—M.—M.—M.—M. Of these, M (the *d* card at the nest) was first choice 14 in 20 times, or about twice as often as to be expected in random selection. However, this might mean merely that the bees were learning to look for their nest at the top of the "L" formation of cards.

Accordingly, at 4:00 P.M. the *a* cards were moved to H and N, leaving the *d* card on *M*, and immediately afterward records were made of twenty returning bees. Sixteen made the correct choice the first time. The four that did not went as follows: N, N, M.—N, N, N, and away.—N, and away.—N, M. The H space was at this time the top of the new "L" formation; and the fact that it was not chosen at all is interesting. Clearly, however, 16 correct first choices in 20, when the random chances are 2 to 1 against such a choice, indicates that either some association had been established between the *d* card and the nest-opening or that the bees had more firmly fixed the relation between the position of the nest-opening and other features of its environment.

The cards were left in this H, M, N formation and at 9:15 A.M. the next day (March 6) previously unused cards were substituted for those which in the preceding day and a half might have acquired some distinctive odor from the bees. Immediately after the change twenty returning bees went directly to the fresh *d* card on M (the nest-opening) with fresh *a* cards in the H and N positions that had been marked with the *a* color-pattern since 4:00 P.M. of the previous day.

However, when at 9:30 I changed the *a* cards to G and L, of twenty returning bees only 5 went to M as first choice and 15 went to L. None went to G at all and only two of those that first went to L repeated that error. Since from 4:00 P.M. of the previous day the correct card had one card above it and one to the right of it, and since, when this arrangement was suddenly changed so that a wrong choice (L) had that position among the three cards, the bees made a first choice of L 15 times in 20, it is clear that they were using as a clue the positions of the cards relative to each other. But, since G was not chosen at all and five correct choices (M) were made, there is an indication of the use of either color or color-pattern as an additional clue.

At 10:30 A.M. the *a* cards were changed to S and T and the behavior of twenty returning bees was recorded. Since there was considerable confusion, the records are given in full: T, M.—M.—S, T, S, M.—S, S, S, S, T, M.—T, S, T, S, T, M.—T, T, T,M.—T, S, M.—T, T, M.—S, S, S, T, S, M.—S, S, S, S and alighted there, T, S, S and alighted, M.—S, T, T, S, T, T, S, M.—S, S, M.—S, S, S and alighted, S, T, M.—S, M.—S, S, S, M.—S, S, T, M.—T, M.—T, T, T, M.—S, T, T, M.—T, T, M. Clearly, in spite of bearing an *a* color-pattern, the favorite choice was S, the card which not only had one above it and one to its right but which was also in the same vertical line in space as the nest.

By 12:55 P.M. the bees had apparently become more accustomed to the arrangement. Of twenty returning bees, 12 went directly to M. The records of the other eight were: T, M.—S, M.—S, T, M.—T, M.—T, T, S, S, M.—S, S, T, M.—T, M.—S, M. The number of correct first choices was well above chance.

At 1:15 P.M, the *a* cards were changed to G and H and immediately afterward twenty returning bees were watched. Of these, 17 went directly to M. The three that did not went as follows: H, G, H, M.—H, M.—G, M. Since the bees had had no time to learn the new arrangement of cards, this is a striking score. It should be noted that, while previously the cards had been arranged so that, looked at as a whole, they formed an "L," in the present arrangement the "L" was reversed and inverted. At 2:55 P.M. with the *a* cards still at G and H twenty bees made the perfect score of going directly to the *d* card at M. This result might be due to an association of the *d* color-pattern with the nest-opening or an orientation with other landmarks or a combination of the two.

It then seemed desirable to have a test in which the color-pattern that had previously marked the location of the nest-opening was placed elsewhere. Accordingly, at 3:15 P.M. a *d* card was placed at R and *a* cards at L and S, the latter being a favored position, as has been noted. These positions adjoin the nest-position. Both cards and folders were previously unused so that there might be no odor-clue. The first bee to return darted seven times at the *d* card on R before locating the nest-opening. The next bee darted twenty-two times at R and the next one twelve times at the same card. The fourth bee darted at the *d* card on R seven times, then tried the *a* card on S once, and then the *d* card on R twice again before locating the nest-opening. The "dartings" of each of these bees sometimes went so far as to become alightings and searchings for the opening that was not there; but the action was too

rapid for me to make more detailed notes. In fact, when I next tried to make a record of an individual bee, there were five bees at once trying to get into the *d* card at R and in the crowd I lost track of the bee I was watching. Fixing my attention on another, I saw it go to R three times, then to S twice, R three times, S five times, and then to the nest. Another went to R ten times, L once, R twice, and then to the nest. Then two at once were repeatedly trying to get into R before they found the nest. Following these, three more bees did the same thing, frequently alighting in the center of the *d* card at R. This behavior of the bees was continued throughout the fifteen minutes of observation. No instance was noted in which a returning bee did not go first and repeatedly to the *d* card, the color-pattern which had for two days marked the location of their nest but which was now falsely placed.

At 3:30 P.M. a *d* card was replaced at M and *a* cards were placed at L and N, making for the first time a linear arrangement of cards. At 6:30 A.M. the next day (March 7) the *a* cards were shifted to R and T; at 8:30 to G and R; at 9:30 to R and V; at 10:30 to A and G; and at 12:30 P.M. to L and N.

At 1:30 P.M. a test was made in which four color-patterns were used: the *a* and *d* of the previous work and also *b* and *c* of figure 2. The *b* card had the pattern of *a* but the white reflected ultraviolet; the *c* card had the pattern of *d* but the white did not reflect ultraviolet. The *a* card was put on T; the *b* one on R; the *c* one on I; and the *d* one on G. The cards thus surrounded the nest-location but avoided it and also both its vertical and its horizontal line in space. All cards and folders were previously unused. Because of the confusion of many bees hovering in front of this new and complicated arrangement, note-taking was difficult. The *c* card at I was the favorite, with the *d* one at G a fair second, then came the *a* card at T, while the *b* card at R was the least visited of all. Apparently the segmental pattern (*c* and *d*) as contrasted with the semicircular one (*a* and *b*) was associated by the bees with their nest-entrance. This was in accord with their three days of training. On the other hand, they selected the cards on which the white did not reflect ultraviolet (*a* and *c*) more often than those on which it did (*b* and *d*), although the cards that had been marking the site of their nest-opening bore the white that reflected ultraviolet. The reason for this is not clear, but apparently the black-and-"white" patterns impressed the bees more than did the difference in the whites.

At 1:45 a *d* card was replaced at M and *a* cards were placed at H and S. At 2:30 one *a* card was changed from S to T, and at 3:30 another test

was made. In this test only two cards were used: a *c* one at S (a favored position) and a *d* one at R. It is to be noted that the *c* card had a segmental black-and-white pattern like that of *d*, which had been marking the nest-opening, but its white did not reflect ultraviolet. The following are the records of returning bees observed immediately after the cards were placed: R, R, R, R, R, nest.—R, R, S, nest.—R, R, R, R, nest.—R, S, R, nest—Three bees now bunched at R and none at S.—S, S, nest.—S, S, S, nest.—R, R, R, S, R, S, R, nest.—R, R, R and then lost in the mass of bees in front of R.—R, R, R, R, nest. Most of the bees that darted at R alighted one or more times.

It seems that, when there is no difference in pattern, the card on which the white reflected ultraviolet (as it did on the nest-card during training) was associated by the bees with their nest. If this be true, not only can the bees be "trained" to form associations but they can distinguish between patterns and also, the patterns being the same, between white that reflects ultraviolet and white that does not. The following experiment was made in order to test more thoroughly this last point.

Distinguishing Ultraviolet

In this experiment *c* and *d* cards were used. The only certain difference between these cards is that the white of *c* reflected little or no ultraviolet and the white of *d* was tinged with that color. Possibly the bees could detect a difference between the odors, if any, of these paints. Each paint was water color, and to humans neither had an odor. The *d* pattern was still used to mark the nest-opening in order that the training effected in the previous experiment might be of at least some use in this one.

At 3:45 P.M., March 7, a *d* card was placed at M and a *c* one at S. At 4:45 the latter was changed to N. The next day at 6:30 A.M. fresh cards were put out, *d* at M, *c* at L, and another *c* at R. Shifts of *c* cards were made as follows: 8:30 N, S; 9:30, C. H.; 10:30, H. R; 11:30, R. V; 12:30, T. V; 1:30, I, T; 2:30, G. I; 3:30, I, S; 4:30, H, S; and 5:30, A, G. March 9: 6:30, M, X; 8:30, I, X; 9:30, I, L; and 10:30, I, M.

At 11:05 the *d* card was placed at L and the *c* cards at G and H. The first choice (the card at which a bee darted on its return from foraging) of twenty bees was, without exception, the *d* card at L. At 11:15 the *c* cards were shifted so that one was at H and another at S, the *d* card still being at L. Of twenty "first choices," 16 were the *d* card at L and 4 the *c* card at S, the square which has previously been noted as favored. See figure 3.

At 11:30 training was resumed with the *c* cards at G and N. At 12:45 a demonstration test was made for and with the coöperation of two friends. It happened that the initials of our last names are respectively H, L, and S. Therefore each man was assigned to watch the square corresponding to his initial and each man selected at random a card for his square. Mr. H. got a *d* one and Messrs L. and S., *c* ones. In ten minutes of watching Mr. H. counted twenty first choices of his *d* card, Mr. L. one on his *c* card, and Mr. S. two on his *c* card. Clearly the bees distinguished between the *d* card on which the white reflected ultra-

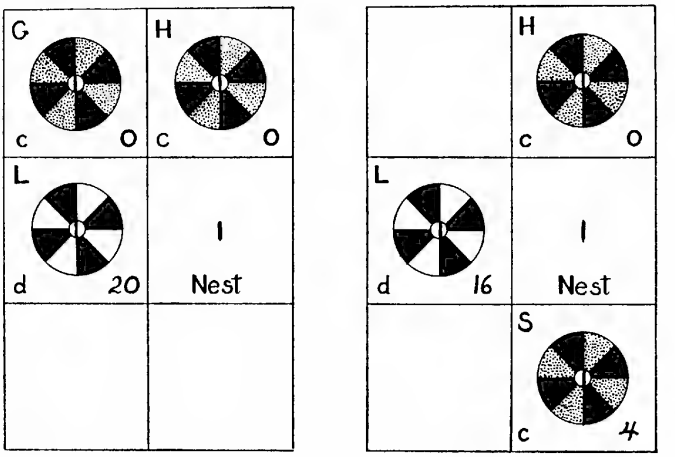


Fig. 3.—Diagrams of two tests involving patterns *c* and *d* (see Fig. 2). Pattern *d* had marked the location of the nest.

violet and the *c* cards on which the white was little or not at all tinged with that color.

People with whom I have discussed this experiment seem prone to slip into the error of thinking that it indicates an attraction of bees to ultraviolet. It does nothing of the kind. There would have been no more difficulty in training the bees to form the opposite association and then, in looking for their nest, they would have avoided ultraviolet. The same error crops up in discussions concerning ultraviolet flowers and flower-visiting insects. Our work did not show that ultraviolet flowers are more attractive. The most that can be said is that ultraviolet is a color to insects just as red is a color to man and as blue is a color to both insects and man. In the following experiment with *Trigona* there was no more ultraviolet on one card than on the other; the cards differed merely in the distribution of that color.

DISTINGUISHING PATTERNS OF WHITE WITH AND WITHOUT ULTRAVIOLET

As my stay in *Trigona* country was to be short, I hastened on to another experiment. The cards used in this were *e* and *f*. These are the same as *b* and *d* respectively except that the black is replaced by a white (Chinese) that reflects little or no ultraviolet (see Fig. 2). To humans the two whites are almost indistinguishable, but by looking closely in a good light we can notice a slight difference in the surface-finish of the two; and the one which reflects ultraviolet is a trifle more cream-colored than the rather chalky Chinese white. It is quite improbable that insect vision is acute enough to perceive the slight difference in texture. The actual differences in humanly visible color-reflection from these paints, as shown by the photometric determinations kindly made by

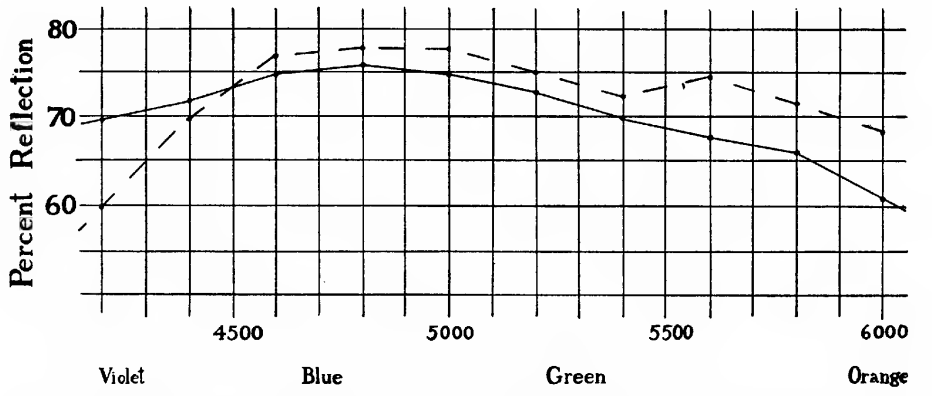


Fig. 4.—Per cent reflection curves between red- and ultraviolet of (solid line) the white paint which strongly reflected ultraviolet and (broken line) the white paint which did not.

Mr. E. N. Grisewood, are so slight in the region between red and ultraviolet (see Fig. 4) that they may be dismissed in a discussion involving insect vision. The difference in ultraviolet is indicated by figure 5.

Supposing that either or both paints had an odor which the bees could detect, it would seem unlikely that there was a difference between the cards in odor, since there were equal areas of each kind of paint on each card. However, if the bees can see ultraviolet, the cards as they saw them differed markedly in pattern, *e* consisting of two semicircles (one strong in ultraviolet; the other not) and *f* consisting of eight segments alternating as to ultraviolet and its absence.

For five days before the starting of this experiment the bees had been under successful training in associating a pattern of alternating

black and white-ultraviolet segments with the location of their nest. Now this training must be counteracted and a new association set up.

Meanwhile, as will appear later, there had been, or was in the process of being, set up another nest-location association: namely, that between the site of the nest and the system of tacks and strings that were used to support the folders and their contained cards, possibly also with other features of the side of the building. It is quite believable that, with the positions of three conspicuous cards constantly shifting in the

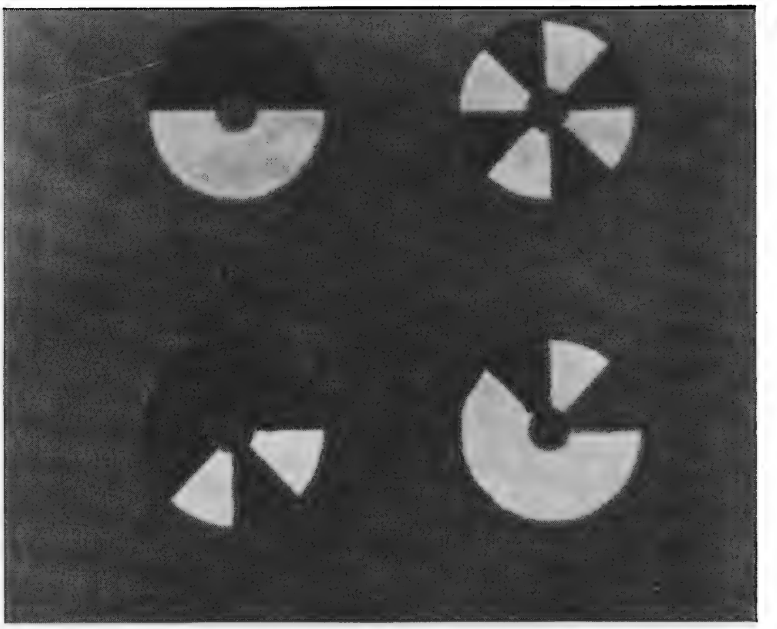


Fig. 5.—A photograph of the all-white patterns *e* and *f* above and *i* and *j* below taken in sunlight through a screen which passed only ultraviolet. The light parts are those areas reflecting ultraviolet.

vicinity of the nest, the bees built up a stronger association between nest-location and less conspicuous but more constant features of the surroundings.

This experiment started at 3:30 P.M., March 9, with an *f* card at the nest (M) and *e* cards at L and S. In what follows, unless the contrary is definitely stated, the *f* card was always at M, the nest-location.

During March 10 the shifts of *e* cards were as follows: 6:45 A.M., G, R; 7:45, G, T; 9:45, S, T; noon, N, T; 1:15, L, N; 3:15, K, L; 4:15, H, S. On March 11 they were: 7:00 A.M., I, L; 8:15, A, V; 8:45,

D, H; 10:00, R, W; 11:00, H, N; noon, N, S; 1:15, L, N; 2:15, K, L; 3:15, N, O; 5:00, N, R. On March 12 they were: 7:00 A.M., G, T; 8:15, T, Z; 9:15, A, G; and 10:15, S, X.

At 11:20, March 12, a test was made with the *f* card at I and the *e* cards at G and S. In this test records were made as accurately as possible of the first definite "dart" at a card made by returning bees. In other words, these records concern the reactions of the bees to the cards as they saw them from a distance of about half a meter. Of twenty bees, 5 darted first at the *f* card on I, 1 at the *e* card on G, and 14 at the *e* card on S. Once again it was evident that there was something about the S location that made it a favorite. Neither G nor I was in line either vertically or horizontally with the nest-opening. Comparing merely G, having an *e* card, with I, having an *f* card, gives an impression that the training to *f* had been at least slightly effective.

The *f* card was then returned to M and the shifting of the *e* cards was continued as follows: 11:40, G, S; 12:30, I, N; 1:30, R, S; 2:30, N, S. At 3:30 a similar test was made with the *f* card at G and the *e* cards at R and T. It will be noted that none of these positions was in either the same vertical or the same horizontal line with the nest. This time there was included in the count the number of bees going directly to the nest, where, during the test, there was no card. Of twenty bees, 12 went directly to the nest, 6 to the *f* card at G, 2 to the *e* card at T, and none to the *e* card at R. Disregarding the clearly strong sense of nest-location and taking into account the fact that there were two *e* cards, the bees seemed to favor the *f* pattern.

The training was then continued with *e* cards shifting as follows: 3:45, V, X; 4:30, C, V; 5:30, A, V. March 13: 6:30, A.M., L, N; 7:30, H, L; 8:30, H, I; 9:30, R, V; 10:30, I, V; 11:30, C, H; 12:30, S, X; 1:40, H, S; and 2:40, L. R.

At 3:00 P.M. March 13, a test was made with fresh cards. An *f* card was placed at G and *e* cards at L and S. Of twenty bees sixteen went directly to the unmarked M, or nest, square; three landed on the *f* card at G; one darted at the *e* card on S; and none at the *e* card on L. The single bee that chose an *e* card went to the one at the favored S square. Three bees going to the single *f* card on an unfavored spot again showed some influence of the training. However, the training was far from complete as was shown when at 3:15 one of the *e* cards was put at M, the other being left at L and the *f* one at G. Of twenty bees, 17 went to M in spite of the *e* card there. Nevertheless, the other 3 went to the *f* card at G and not to the *e* one at L.

This method of scoring leaves much to be desired. It does not take into account the number of bees hovering in front of each of the cards as though inspecting them before either passing on or else darting in to landing. At any instant in the 3:00 P.M. test rather more bees were hovering in front of S than there were in front of either of the other cards, but those individuals that did hover in front of the *f* card at G did so much more persistently. There was very little hovering in front of the *e* card at L.

In order to upset, if possible, whatever it was that was betraying so effectively the position of the nest or, at least, to provide a conspicuous and movable landmark other than the colored cards, the plan was adopted of keeping out nine folders arranged to form a square, three of these folders carrying colored cards and the other six blank. At 4:15 a square of these folders was placed with the corners at G, I, R, and T. An *f* card was put at I and *e* cards at H and L for an immediate test. Once again, of twenty bees, seventeen went first to M, the nest-square now covered, except for the central hole, with a cardless folder. This shows that whatever it was that was guiding the bees to the nest was not masked by the new feature of the nest's surroundings. The other three bees went to the *f* card and there was a very pronounced hovering in front of this but scarcely any in front of either of the two *e* cards.

At 5:45 the square of folders was shifted so that its corners were at B, D, L, and N, bringing the nest-space with an *f* card in the middle of the lower line. The *e* cards were on H and L. The next day, March 14, training was continued with shifts of *e* cards as follows: 7:45, G, H; 8:30, G, I; 9:30, B, H; 10:30, L, N; 11:30, C, I; 12:30, I, N; and 2:00, H. I.

At 2:50 the square of folders was shifted so that the corners were at K, M, V, and X. This gave the W space the same position, relative to the square of folders, that the nest had occupied earlier in the day. K was in the same horizontal line as the nest. R had neither of these advantages and was not in the same vertical line as the nest. An *e* card was placed on each of W and K, and an *f* card was placed on R. The method of scoring was changed so as to take more account of the hovering. In this method I shut my eyes for about five seconds, opened them, quickly noted the positions of bees hovering close to or alighted on cards, then shut my eyes again to repeat the series of observations. Of twenty bees observed in this way, 16 were at the *f* card on R and 2 at each of K and W, the *e* cards. At 3:05 another test was made, using the same method but with an *f* card at W and *e* cards at L and R. This time 13 bees were noted at the *f* card on W, 7 at *e* on R, and 2 at *e* on L. Why

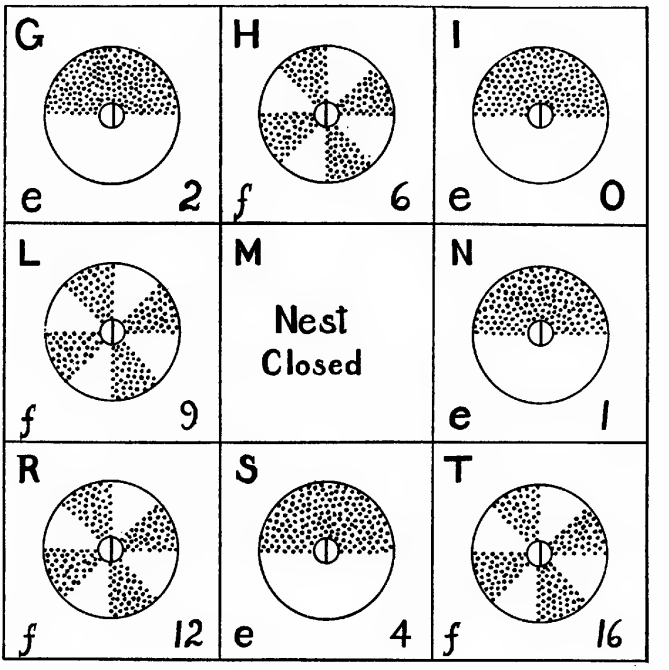
the R space with its *e* card attracted so much attention is not clear unless it was because that had been the position of the *f* card only a few minutes before. At any rate, the *f* card on W received more attention than would be expected on pure chance. The fact that the *f* card did better on the 2:50 test than it did on the 3:05 one, in spite of its apparently better position (with respect to the square of folders) on the second test, may be due to clouds obscuring the sun during the latter test. The bees were called upon to distinguish ultraviolet patterns and quite possibly this is easier in bright sunlight than in dull. Adding the scores of the two tests, the *f* card received 29 and the two *e* cards a total of 13, a ratio of about 4.5:1 in favor of *f*.

The training was then resumed with the folders making the B-D-L-N square and *e* cards shifting as follows: 3:15, H, L; 4:30, C, H. March 15: 8:00, I, L; 9:00, C, L; 9:30, G, I; 10:00, G, N; 11:00, B, G; noon, C, G; 12:30, G, L.

At 1:15 the folders were shifted to cover the square C-E-M-O and fresh cards were used for testing, an *f* one on N and *e* ones on H and I. If the bees had come to use the square of folders as a locality mark, N would be a favored square; and H was in the same vertical line as, but above, the nest. By the method of scoring adopted the day before, N with its *f* card scored fourteen; H, three; and I, one. Then the cards were shifted so that the *f* card was at O and *e* cards at J and N. This time the *f* card scored twenty; the *e* card at N, eighteen; and the *e* card at J, three. Considering only the first of these two tests, one can not say whether N scored so highly because it had the *f* card or because the bees had fixed upon the middle of the lower row (the location of the nest during training; the location of N in the test) of the square of folders as the location of the nest. However, the results of the second test indicate that both of these factors were working.

At 1:40 the folders were placed to cover the square G-I-R-T and training resumed. In this new arrangement of folders the nest-space (M) was the central one. The *e* cards were shifted as follows, *f* still marking the nest: 1:40, N, S; 2:30, L, S; 4:30, R. S. March 16: 7:30 A.M., G, T; 8:10, N, T; 9:00, L, N; 10:15, N, R; 11:00, R, S; noon, H, L; 1:45, I, N; 3:15, I, T. March 17: 7:00, A.M., R, T; 8:00, H, R; 9:30, R, S; 10:00, N, S; 10:50, I, N; noon, H, I; 1:10, I, R; and 2:10,R, S.

At 3:00 P.M., March 17, four *e* and four *f* cards, all previously unused, were put in the folders as shown in figure 6. The nest-opening was closed by a sheet of pasteboard put in the M folder. The scores are shown in the respective spaces. At 3:15 the pasteboard was removed from the



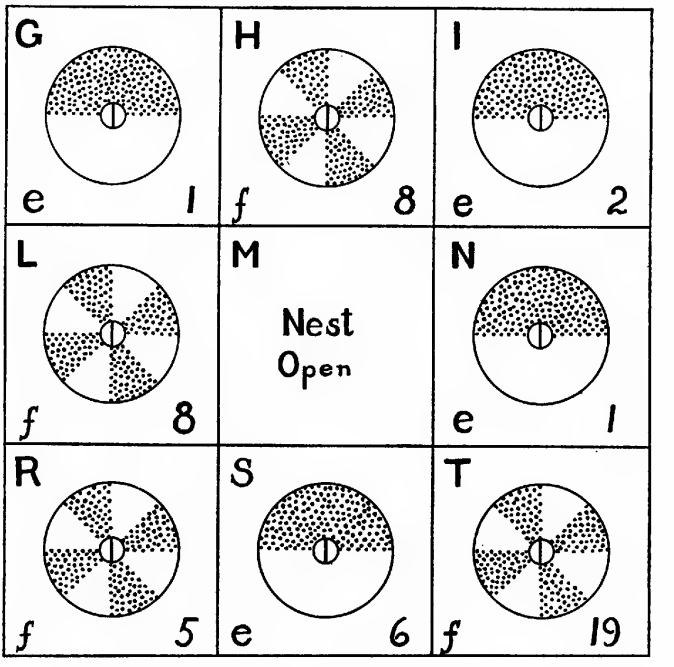


Fig. 6.—Diagrams of two tests involving patterns *e* and *f* (see Figs. 2 and 5). Pattern *f* had marked the location of the nest.

nest-space and the scores recorded as shown in figure 6. There were equal numbers of *e* and of *f* cards so that, disregarding the favored position of the *e* card on S, the random chances were equal that a bee would be at either kind of card. However, there were about five times as many (83:17) at the *f* cards.

Starting at 3:30 a plan was adopted of having *e* cards on each of four, instead of only two, spaces that did not contain the nest. The folders were arranged as before (G-I-R-T) and an *f* card was on M. The *e*

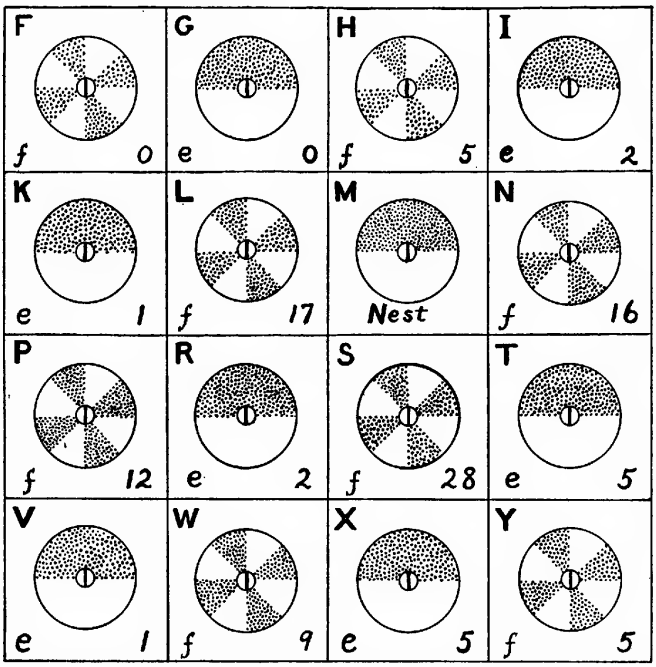


Fig. 7.—Diagram of a test involving patterns *e* and *f* (see Figs. 2 and 5). Pattern *f* had marked the location of the nest.

cards were shifted as follows: 3:30, G, I, N, S. March 18: 7:30, A.M., N, R, S, T; 10:15, G, H, I, L; 2:00, G, H, L, R; 5:00, G, I, R, T. March 19: 8:00 A.M., H, L, N, S; 9:00, L, N, R, T; 10:00, H, I, R, T; 11:30, G, H, I, N; 1:15, G, L, N, T; 2:10, G, L, N, S; and 2:45, H, I, R, S.

Since M, the nest-space, had been in the center of the 3×3 square of folders in the preceding training, the following test was made with a 4×4 square of folders in which no one folder was in the exact center (see Fig. 7). It will be noted that an *e* card was placed at the nest and an *f*

card on S. Most of the bees coming to the *e* card on M hesitated very noticeably before entering and many turned to look elsewhere before doing so. However, once they alighted at M, they were able to go inside and so, being no longer visible, were not counted when scoring that row. Consequently, the M space had better be omitted in considering the scores. This leaves 8 *f*'s to 7 *e*'s; but the *f*'s scored 92 to 16 for the *e*'s, or 5.8:1 instead of the 1.1:1 to be expected on a chance distribution. If we omit the *f* card on S, leaving an equal number of *e*'s and *f*'s, the score was 64 *f*'s to 16 *e*'s, or 4:1 instead of 1:1. This test was made with fresh cards so that no possible odor left by the bees during training would affect the results.

Is the Lower Part of the Pattern More Important than the Upper?

Training was continued the following day (March 20) in the same manner as before, with the folders making the G-I-R-T square and the *e* cards shifted as follows: 7:00 A.M., G, H, R, T; 8:00, H. L, R, T; 9:00, G, I, R, T; 10:00, H, I, R, S; 11:30, G, H, N, S; 12:30, I, L, N, T; 1:00, G, L, N, S; 1:40, G, L. R, T; and 2:10, H, I, N, S.

Having become confident that the bees could distinguish *e* from *f* and that many of them had been at least partially trained to associate *f* with the nest-opening, I wished to determine whether *Trigona*, like *Apis*,[1] paid more attention to the lower part of a pattern surrounding an entrance-hole (in the case of the *Apis* experiments, the entrance to food) than to the upper part. Accordingly, cards with *i* and *j* patterns (Figs. 2 and 5) were used in a test. In *i* the upper half was the pattern (*e*) that during training had been placed where there was no nest-opening, but the lower half was the *f* pattern that during training had been placed at the space (M) containing the nest-opening. The *j* card was just the reverse, the upper half being the "correct" pattern and the lower half the "wrong" one.

The results (see Fig. 8) were quite striking. Omitting the M score for reasons already stated, the chance distribution of the 110 scores would be *e*, 22.0: *f*, 29.3; *i*, 29.3; *j*, 29.3; but the observed scores were *e*, 3: *f*, 50: *i*, 49: *j*, 8. Both the *f* and the *i* patterns were clear favorites, with *i* doing as well as *f* even though only its lower half had the "correct" pattern and its upper half was "wrong." In fact, the score almost "proves too much" because, if we discount the *f* score because one *f* card was in the favored S position, *i* really did better than *f*.

[1]Baumgärtner, *loc. cit.*

If we bear in mind that these scores represent the reactions of a random sample of individuals in a large colony, including "callows" that had emerged since the training started, there seems to be no doubt as to either (1) the ability of the bees to distinguish the to us invisible

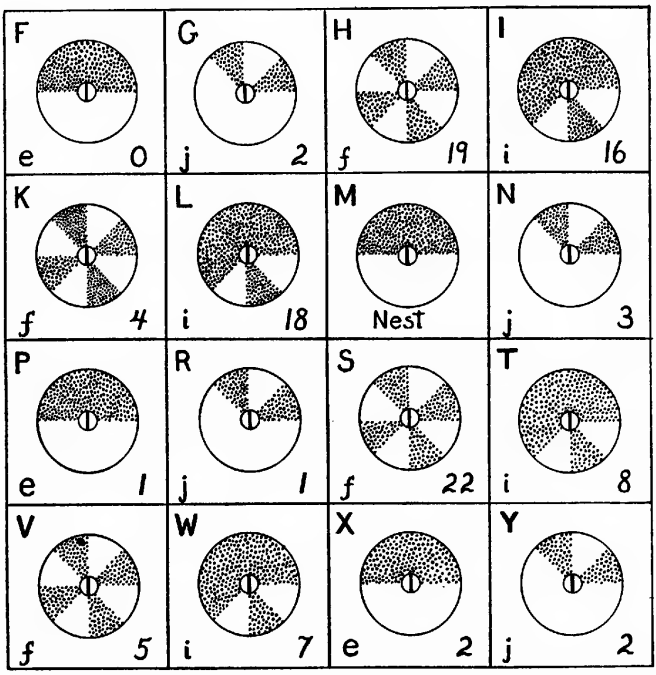


Fig. 8.—Diagram of a test involving patterns *e*, *f*, *i*, and *j* (see Figs. 2 and 5). Pattern *f* had marked the location of the nest.

ultraviolet component of the patterns, or (2) the effect of the training in associating an ultraviolet pattern with such an important feature in their lives as the location of their nest, or (3) that the bees pay attention on alighting (whatever they may do at a distance) only, or at least chiefly, to the lower half of the immediate surroundings of their alighting place.

How Small an Area is Noticed by the Bees?

Training was continued with the *f* pattern still at the nest but the folders and *e* cards shifted as follows. March 20 (folders: G-I-R-T): 3:50, H, I, R, T; 5:00, L, N, R, T. March 21 (folders: G-I-R-T): 7:00, A.M., H, R, S, T; 8:30, I, L, N, S; 9:30, G, L, N, R; 10:10, G, I, S, T; 11:00, H, N, R, T; 11:30, G, H, I, T; 12:20, G, I, L, R; 1:10,

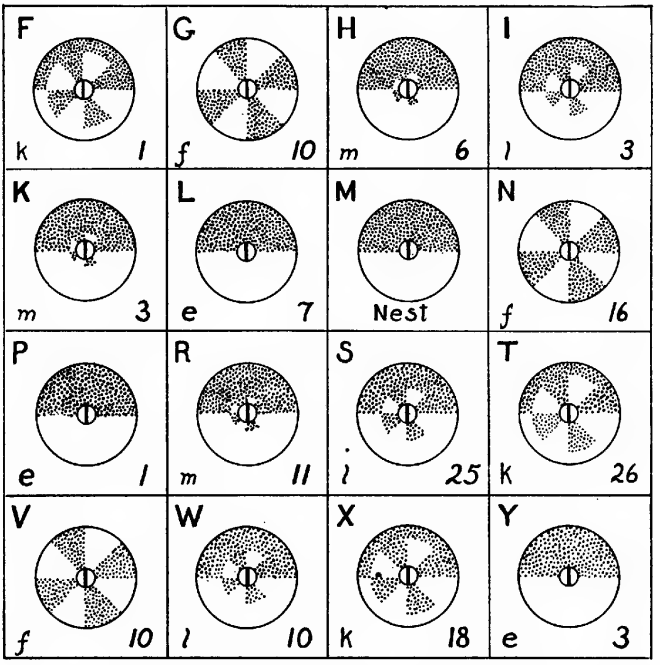


Fig. 9.—Diagram of a test involving patterns *e*, *f*, *k*, *l*, and *m* (see Fig. 2). Pattern *f* had marked the location of the nest.

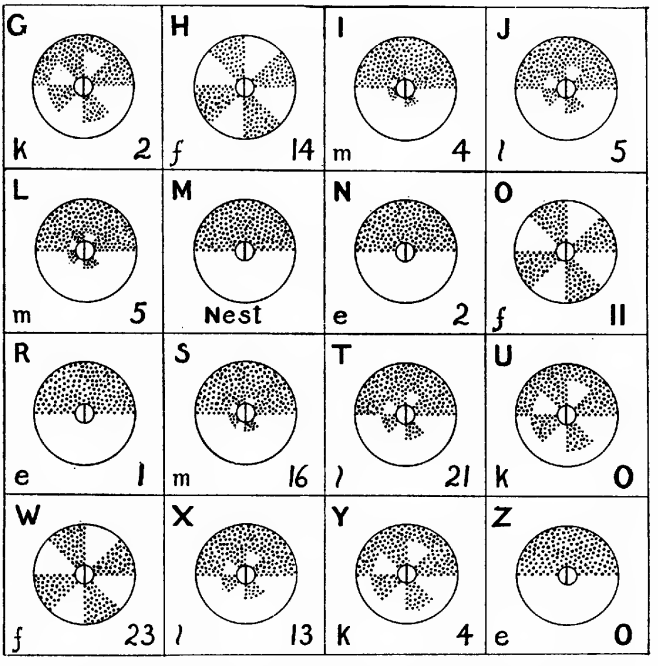


Fig. 10.—Diagram of a test involving patterns *e*, *f*, *k*, *l*, and *m* (see Fig. 2). Pattern *f* had marked the location of the nest.

H, L, N, S; 1:30 (folders, F-H-P-S), H, K, L, S; 2:00 (folders, A-C-K-M), C, H, K, L; 2:30 (folders, C-E-M-O), C, H, I, J.

Then at 3:05, March 21, a test was made with *e*, *f*, *k*, *l*, and *m* patterns (see Fig. 2). The total radius of these patterns was, as before, about 31 mm., and the radius of the central hole was about 7 mm. That is, each card was colored for a distance of about 24 mm. from the edge of the central hole to the outer edge of the pattern. In *k* there was an *f* pattern for 16 mm. from the central hole and then there was an *e* pattern; *l* was the same except that the *f* pattern extended for only 8 mm.; and *m* was the same except that the *f* pattern extended for only abut 4 mm. At the time of the test the sky was very cloudy, presumably with the result that there was very little ultraviolet light. The scores are shown in figure 9. Not counting the *e* card at M, there were three cards of each of the five patterns and 150 positions of bees were noted. On the basis of pure chance we might expect about 30 scores for each pattern, but *e* had 11; *f*, 36; *k*, 45; *l*, 38; and *m*, 20.

Training was then continued with the folders forming the G-I-R-T square, *f* on M, and *e* cards shifting as follows. March 21: 3:30, I, L, R, S; 5:00, G, L, N, R. March 22: 7:45, A.M., G, H, L, T; 8:30, G, H, I, S; 9:05, H, I, N, R; 9:40, I, L, N, S; 10:35, G, N, R, T; 11:25, H, L, S, T; 12:25, G, I, R, S; 12:55, H, L, N, R; and 1:30, G, I, L, T.

A second test was made at 1:45, March 22, with better light. The position of folders and cards, also the individual scores, is shown in figure 10. The total scores were *e*, 3; *f*, 48; *k*, 6; *l*, 39; and *m*, 25. Since 121 positions were noted, the expectation on the basis of chance was about 24 for each pattern. Why *k* scored so low is not clear.

Combining the scores of the two tests, we find *e* with 14; *f*, 84; *k*, 51; *l*, 77; and *m*, 45. Apparently *m*, with only the small inner part (a band 4 mm. wide around the central hole) bearing the *f* pattern and all the rest bearing the *e* pattern, was accepted to an appreciable extent by the bees as not being the *e* pattern. Unfortunately, my stay at the Laboratory was then nearly over and I neither determined this point more accurately nor even attempted to determine from what distance the bees discovered that *m* was not *e*.

I did, however, take up patterns *h*, *n*, and *o* (see Fig. 2). The *h* pattern is a combination of *f* and *g*. In *n* the lower half was like the lower half of *l*, but the upper half was like the upper half of *e*. In *o* it was the lower half that was like the corresponding part of *e*, and the upper half was like the upper half of *l*. On the basis of what had gone before it was expected that the bees would to some extent accept *n* as

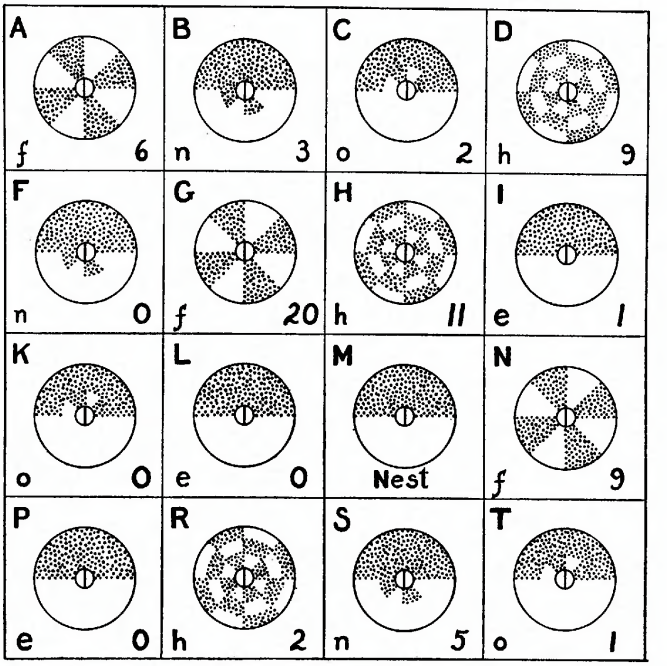


Fig. 11.—Diagram of a test involving patterns, *e*, *f*, *h*, *n*, and *o* (see Fig. 2). Pattern *f* had marked the location of the nest.

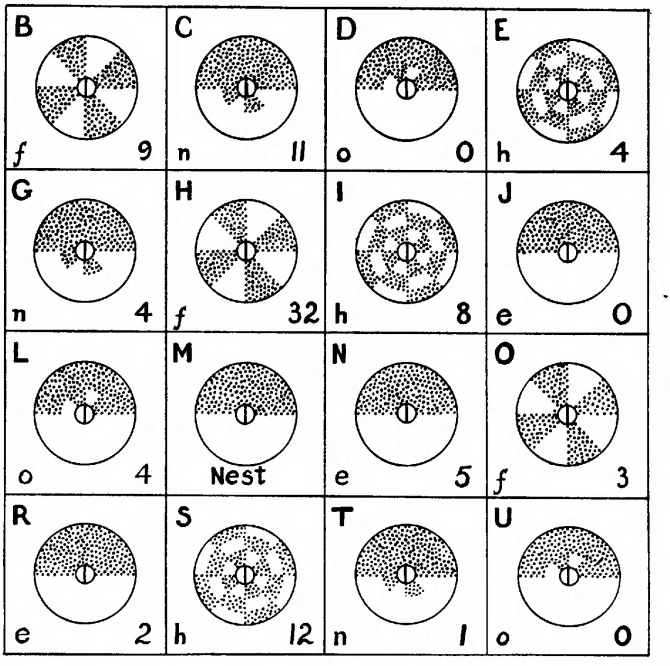


Fig. 12.—Diagram of a test involving patterns *e*, *f*, *h*, *n*, and *o* (see Fig. 2). Pattern *f* had marked the location of the nest.

the pattern marking their nest, because *n* had the *f* pattern for a distance of 8 mm. from the edge of the lower half of the central hole. For a similar reason it was expected that they would not favor *o*. Since *h* was exactly like *f* for some distance around the circular hole and resembled *f* elsewhere it was expected that *h* might be more favored than *n*.

The bees were given a short period of renewed training with the folders covering the M-O-X-Z square, an *f* card on M, and *e* cards shift-

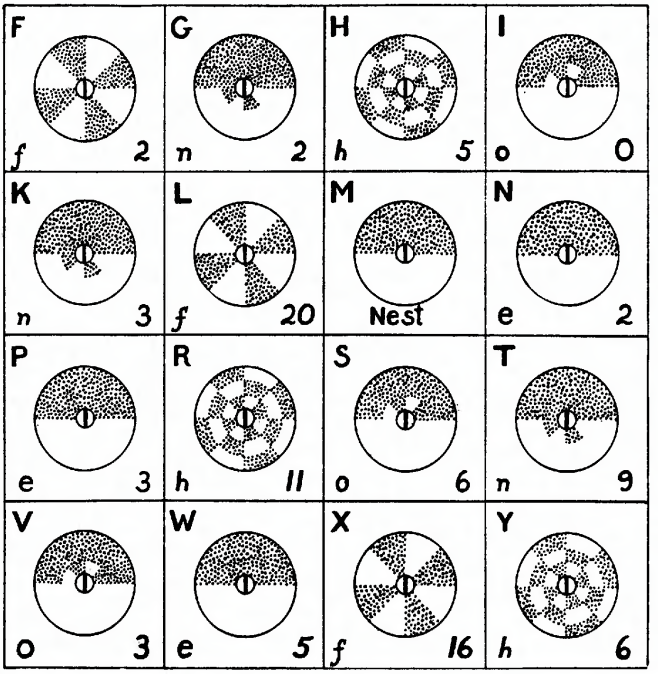


Fig. 13.—Diagram of a test involving patterns *e*, *f*, *h*, *n*, and *o* (see Fig. 2). Pattern *f* had marked the location of nest.

ing as follows. March 22: 2:30, N, S, T, Y; 3:00, O. S, T, Z; 4:30, S, T, Y, Z. March 23: 7:30, N, U, X, Y; and 8:15, N, S, T, U.

Then a test was made at 10:20 and again at 10:40, March 23, with folders, cards, and results as shown in figures 11 and 12. After these tests the folders were put on the B-D-L-N square, and *f* card on M, and *e* cards shifted as follows: 11:00, G, I, L, N; 12:15, C, G, H, I; 1:40, D, H, I, N; and 2:20, B, G, H, L. From 3:00 to 4:00 various simple demonstration tests were made for visitors at the Laboratory and at 4:00 the test diagrammed in figure 13 was made without preliminary training. Adding the results of these three tests together we find that *e* cards scored 18; *f*, 117; *h*, 68; *n*, 38; and *o*, 16.

These results fairly well met expectations, although *f* scored rather more heavily than was anticipated. This strong favoring of *f* so decreased the scores on the other patterns that, in considering them, the comparison should be made with *e*, basing the calculation on the total number less *f*. Possibly the bees, fooled by so many tests, had taken to looking more carefully at the whole pattern from a distance before coming closer.

SUMMARY

The social, tropical bee, *Trigona cressoni parastigma*, could not be induced to come for any of a variety of substances that were offered as food in order to test its ability to form an association between the location of food and some color or color-pattern. However, it was found possible to confuse this bee as to the location of its nest by associating a color or color-pattern with the nest-site and then moving that color or color-pattern elsewhere.

When a pattern of alternating segments of black and white was used, the bee distingished between white tinged with ultraviolet and white not so colored. It also distinguished patterns made up of white with ultraviolet and white without that color. In the training from March 9 to 23 *f* had marked the site of the nest and one or more *e* cards had been at various other positions in its vicinity (see Figs. 2 and 5). The frequent "tests" probably tended to break down to some extent this training because in them bees going to an *f* card found that at that time *f* did not mark the site of the nest but that sometimes *e* did. Furthermore, these tests presumably included bees that recently emerged and, so, had not been subjected to full training. In spite of these unfavorable factors, a comparison of the observed scores with numbers expected on the basis of pure chance show that in all of the seventeen tests (see Table 1), except the first, *f* exceeded expectation. Combining all of the tests, *f* scored 506 and *e* scored 124, a ratio of 4.1 to 1, whereas chance would have given a ratio of only 0.8 to 1.

Whatever may be this insect's reactions at a greater distance, when alighting it is influenced most by the appearance of a band 4 to 8 mm. wide along the lower half of its entrance hole, this being the place on which it actually alights.

The fact that this feral species in its natural environment appreciates and reacts to reflected ultraviolet of sunlight adds to the importance of considering the ultraviolet color of many flowers when discussing the relation between floral color and the behavior of flower-visiting insects.

TABLE I.—Summary of tests in which all of the cards appeared to humans to be almost uniformly white but each of the cards had a distinctive pattern in ultraviolet (see Fig. 2). During training the *f* card had marked the location of the nest.

	Expectation on Basis of Chance										Observed Scores										
	e	*f*	*h*	*i*	*j*	*k*	*l*	*m*	*n*	*o*	*e*	*f*	*h*	*i*	*j*	*k*	*l*	*m*	*n*	*o*	Totals
March	13.3	6.7	....	....	....	....	....	....	.....	....	15	5	..	..	..	..	..	..	..	..	20
12	5.3	2.7	....	....	....	....	....	....	....	....	2	6	..	..	..	..	..	..	..	..	8
13	2.6	1.3	....	....	....	....	....	....	....	....	1	3	..	..	..	..	..	..	..	..	4
	2.0	1.0	....	....	....	..	....	....	....	....	0	3	..	..	..	..	..	..	..	..	3
14	13.3	6.7	....	....	....	....	....	....	....	....	4	16	..	..	..	..	..	..	..	..	20
	14.7	7.3	....	....	....	....	....	....	....	....	9	13	..	..	..	..	..	..	..	..	22
15	12.0	6.0	....	....	....	....	....	....	....	....	4	14	..	..	..	..	..	..	..	..	18
	27.3	13.7	....	....	....	....	....	....	....	....	21	20	..	..	..	..	..	..	..	..	41
17	33.3	16.7	....	....	....	....	....	....	....	....	7	43	..	..	..	..	..	..	..	..	50
	33.3	16.7	....	....	....	....	....	....	....	....	10	40	..	..	..	..	..	..	..	..	50
19	50.4	57.6	....	....	....	....	....	....	....	....	16	92	..	..	..	..	..	..	..	..	108
20	22.0	29.3	....	29.3	29.3	....	....	....	....	....	3	50	..	49	8	..	..	..	..	..	110
21	30.0	30.0	....	....	....	30.0	30.0	30.0	....	....	11	36	..	..	..	45	38	20	..	..	150
22	24.2	24.2	....	....	....	24.2	24.2	24.2	....	....	3	48	..	..	..	6	39	25	..	..	121
23	13.8	13.8	13.8	....	....	....	....	....	13.8	13.8	1	35	22	..	..	..	..	..	8	3	69
	19.0	19.0	19.0	....	....	....	....	....	19.0	19.0	7	44	24	..	..	..	..	..	16	4	95
	18.6	18.6	18.6	....	....	....	....	....	18.6	18.6	10	38	22	..	..	..	..	..	14	9	93
Totals	335.1	271.3	51.4	29.3	29.3	54.2	54.2	54.2	51.4	51.4	124	506	68	49	8	51	77	45	38	16	982